DEVELOPMENT AND VALIDATION OF STABILITY INDICATING RP-HPLC METHOD FOR THE ESTIMATION OF ZIPRASIDONE IN CAPSULE DOSAGE FORM

Banerjee Bhaskar, Shrivastava Pratibha*, Shailendra Singh Panwar and Tanushree Banerjee
Department of Pharmaceutical Chemistry, RKDF College of Pharmacy, Hoshangabad road, Misroad, Bhopal, M.P, India

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ABSTRACT
A sensitive, reproducible and rapid reverse phase high performance liquid chromatographic methods were developed and validated for the determination of Ziprasidone Hydrochloride Monohydrate (ZHM) in the presence of its degradation products in pure form and in pharmaceutical formulations. Hypersil C\textsubscript{18} column (150×4.6mm, 5\textmu m) was used with a mobile phase containing a mixture of 0.02M KH\textsubscript{2}PO\textsubscript{4} (pH-3), Buffer : Methanol : TEA in the ratio of (70:30:0.1). The flow rate was 1.8ml/min and effluents were monitored at 315nm and eluted at 6.204min. Calibration curve was plotted with a range from 35-65 \mu g/ml. The assay was validated for the parameters like accuracy, precision, robustness and system suitability parameters. The proposed method can be useful in the routine analysis for the determination of ziprasidone in pharmaceutical dosage forms.

KEY WORDS: Ziprasidone, Reverse phase HPLC, Pharmaceutical dosage forms

INTRODUCTION
Ziprasidone, a benzothiazolylpiperazine derivative, is an FDA approved atypical antipsychotic agent to treat psychiatric conditions such as schizophrenia, hallucinations, delusions, hostility and other bipolar disorder. The oral form of Ziprasidone is the hydrochloride salt, Ziprasidone hydrochloride. Ziprasidone has a high affinity for the dopamine D2 and D3, the serotonin 5HT2A, 5HT2C, 5HT1A, 5HT1D and alpha 1-adrenergic receptors\textsuperscript{4}, and moderate affinity for the histamine H1 receptor where it is believed to act as an antagonist.

Ziprasidone (ZPR) is chemically known as 5-[2-[4-(1,2-benzisothiazol-3-yl)-ethyl]-6-chloro-1,3-dihydro-2H-indol-2-one.

Figure 1. Chemical Structure of ZHM

Comprehensive literature survey reveals that several analytical methods have been reported for the estimation of ZHM\textsuperscript{2} which includes Reverse Phase High Performance Liquid Chromatographic [RP-HPLC]\textsuperscript{3,4}, High Performance Thin Layer Chromatography [HPTLC]\textsuperscript{5}, UV-Visible Spectrophotometry \textsuperscript{6,7,8}, Fluorescence \textsuperscript{9} and Electrochemistry\textsuperscript{10}. Most of them are based on visible spectrophotometric methods, LCMS \textsuperscript{11,12}, UPLC-MS\textsuperscript{13}, NPHPTLC \textsuperscript{14}, HPLC-MS, and Mass\textsuperscript{15}. The objective of the present work was to develop a rapid and accurate HPLC method with UV detection in bulk drug and its dosage forms. The developed method was simple, precise, sensitive and very useful for the determination of ziprasidone in bulk and pharmaceutical dosage form.

MATERIAL AND METHODS

Chemicals
Ziprasidone standard, Active pharmaceutical ingredient (API) and capsules formulation containing 80mg of ziprasidone were supplied by Zydis Cadila Healthcare Ltd. Moraiya (Guj). HPLC grade methanol and water as well as potassium dihydrogen phosphate, A.R. grade were purchased from Merck, Mumbai, India. All other chemicals used were of HPLC grade or A.R. grade.

Instrumentation
The HPLC system Consisted of a Shimadzu Class LC- 10AT vp module with auto injector (SIL-10 ADvp) and UV-VIS & PDA detector (SPD-10 Avp). The data acquisition was performed by class-vp 6.14 software.

Chromatographic conditions
The mobile phase used in this study was a mixture of Buffer: Methanol and TEA (70:30:0.1) phosphate buffer (pH-3) . Stationary phase was Hypersil BDS C\textsubscript{18} (150×4.6mm,5\textmu m) dimensions at ambient temperature. The contents of the mobile phase were filtered before use through a 0.45\textmu m membrane. The mobile phase was pumped at a flow rate of 1.8ml/min for 8min. The elute was monitored at 315nm using UV-detector. The injection volume was 10 \mu L. The retention time of the drug was found to be 6.240 min.

Preparation of standard drug solutions
The standard stock solution of the ZHM was prepared by dissolving 57mg of pure ZHM in 100ml of diluents to give the final concentration of 500\mu g/ml. The solution was sonicated for 30 minutes to insure the dissolution of ZHM. The working standard solution of ZHM was prepared by taking suitable aliquots of drug solution from the standard stock solution and the volume was made up to 50 ml with diluents to get concentrations of 50\mu g/ml. The solutions were filtered through 0.45\textmu m membrane filter before injection and 20\mu l solution was injected in six replicates to the chromatographic system.

Preparation of sample solutions
For the preparation of sample solutions, twenty capsules were weighed, powder was collected and mixed. A quantity equivalent to 50 mg of ZHM was transferred into extraction flask, to this suitable amount of diluents (Water: Methanol;
2:3) was added and the mixture was subjected to sonication for 30 min for complete extraction of drug. The solution was filtered into 100ml volumetric flask and made up to the mark with diluent. From this, different aliquots were taken in separate 50ml volumetric flasks. The contents of the flasks were made up to the volume with mobile phase to get 50μg/ml concentration and mixed well. The solutions were filtered through 0.45μ membrane filter before injection and 10μl solution was injected in six replicates to the chromatographic system.

**Method validation**

The method was validated by International Conference on Harmonization (ICH) guidelines for linearity, range, precision, specificity, accuracy, LOD, LOQ, ruggedness, robustness and solution stability, parameters.

**RESULTS AND DISCUSSION**

**HPLC method development and optimization**

The chromatographic method was optimized by changing various parameters, such as the mobile phase composition, pH of the buffer used in the mobile phase. Retention time and separation of peak of ZHM were dependent on pH of the buffer and the percentage of methanol. Different mobile phases were tried, but satisfactory separation and good symmetrical peak for Ziprasidone and its degradation product were obtained with the mobile phases consisting of Buffer: Methanol and TEA (pH-3) in the ratio of (70:30:0.1). Various reversed-phase columns were used but the Hypersil C₈ column (150×4.6mm,5μm) gave the minimum elution time with good resolution. A representative chromatogram is shown in Figure 2.

![Figure 2. A typical chromatogram showing the peak of ziprasidone](image)

**System suitability**

For system suitability, six replicates of standard solution were injected and studied the parameters like theoretical plates, theoretical plates per meter, tailing factor (k) and Height Equivalent Theoretical Plate. The represented data was shown in Table no 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Ziprasidone HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak area</td>
<td>209450.167</td>
</tr>
<tr>
<td>No. of theoretical plates</td>
<td>2110.52</td>
</tr>
<tr>
<td>Retention time (min)</td>
<td>6.23</td>
</tr>
<tr>
<td>Asymmetry</td>
<td>1.90</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Specificity**

The HPLC chromatograms recorded for the placebo showed almost no peaks within a retention time range of 8min. The peak for ZHM is clearly separated from other excipients of the formulation. The retention time, tailing factor and peak area of the ZHM in marketed formulations and in dissolution samples of capsules were not affected with excipients present in formulations as well as with capsule cells, indicating a high degree of specificity of this method. Thus, the HPLC method presented in this study is specific for ZHM.

**Linearity**

The standard curve was obtained in the concentration range of 35-65μg/ml. The linearity of this method was evaluated by linear regression analysis. Slope, intercept and correlation coefficient [r²] of standard curve were calculated and given in Figure no 3 to demonstrate the linearity of the method.

![Figure 3. Calibration curve for ZHM](image)

**Accuracy**

To check the accuracy of the method, recovery studies were conducted after addition of standard drug solution at three different levels i.e. 50 %, 100 %, and 150 % to pre-analyzed sample solution. The results are given in Table no 2.

<table>
<thead>
<tr>
<th>Amount of pure ZIP added to the placebo in μg/ml</th>
<th>Amount of ZHM found in μg/ml Mean ± SD</th>
<th>% Recovery</th>
<th>% RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 (70%)</td>
<td>34.91</td>
<td>100.4</td>
<td>0.3</td>
</tr>
<tr>
<td>50 (100%)</td>
<td>49.62</td>
<td>98.7</td>
<td>0.7</td>
</tr>
<tr>
<td>65 (130%)</td>
<td>64.60</td>
<td>99.0</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**Method precision**

The method precision was done by preparing six different sample preparations by one analyst under the same conditions and determining the assay of these samples. The mean area of six determinants was found to be 98.3 and percentage RSD was 0.5.

**Intermediate Precision: (Ruggenedness)**

Intermediate precision was carried out to demonstrate the reproducibility of test results obtained by this method for variability namely instrument to instrument, column to column, analyst to analyst and day to day. The results are given in Table 3.
Application of Stress (Forced Degradation Study)
To perform the forced degradation study, 50 mg drug was subjected to acidic, alkaline, oxidizing, thermal and heat & moist conditions. For acidic degradation the drug was heated with 5 N HCl at 80°C for 2 h, for alkaline degradation the drug was heated with 5N NaOH at 80°C for 2 h, for degradation under oxidizing conditions the drug was exposed with 10% (v/v) H₂O₂ at room temperature, for thermal degradation the drug solution was exposed at 100°C for 24 h and for heat & moist degradation the drug solution was exposed to 100°C for 48 h. The placebo was also subjected to the same stress conditions to determine whether any peaks arose from the excipients or not. After completion of the treatments the solutions were left to return to room temperature and diluted with water: methanol (40:60, v/v) to furnish 50 µL/mL solutions. The drug was gradually decreased with time on heating at 80°C in 5N HCl, forming major degradation products at RRT 3.94 (Figure 4). Under alkaline condition the drug degrades by the decreased areas in the peaks when compared with peak areas of the same concentration of the non-degraded drug, without giving any additional degradation peaks (Figure 5). Drug degradation was associated with rise in a major degradation product at RRT 3.94 and the drug shows three additional peaks in hydrogen peroxide (30%) degradation at tₘ 5.27, 7.57 and 14.79 min (Figure 6).The drug was stable to thermal condition at 70°C for 72 h and heat and moist condition. The results are shown in

**Table 3: Precision data of ziprasidone**

<table>
<thead>
<tr>
<th>Observation</th>
<th>Ziprasidone HCl(%)Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analyst 1 (Instrument 1)</td>
</tr>
<tr>
<td></td>
<td>Column 1</td>
</tr>
<tr>
<td>Mean %Assay</td>
<td>98.5</td>
</tr>
<tr>
<td>% RSD</td>
<td>0.23</td>
</tr>
</tbody>
</table>

**LOD & LOQ**
The limit of detection (LOD) and limit of quantitation (LOQ) was 0.66µg/ml and 1.83µg/ml respectively.

**Robustness**
The percentage recovery of ZHM was good under most conditions and didn’t show any significant change when the critical parameters were modified. The tailing factor for ZHM was always less than 2.0 and it was well separated under all the changes carried out. Considering the modifications in the system suitability parameters and the specificity of the method it can conclude that the method is robust.

**Application of the method to pharmaceutical dosage forms**
The method is sensitive and specific for the quantitative determination of Ziprasidone and also subjected to validation for different parameters, hence has been applied for the estimation of drug in pharmaceutical dosage forms. Capsules from two different manufacturers were evaluated for the amount of ZHM present in the formulations. Each sample was injected six times after extracting the drug as mentioned above in experimental section. The amount of ZHM was found to be within the range of 99.85%-100.1%. None of the capsule excipients were found to interfere with the analyte peak and the results were shown in Table no 4.

**Application of Stress (Forced Degradation Study)**
To perform the forced degradation study, 50 mg drug was subjected to acidic, alkaline, oxidizing, thermal and heat & moist conditions. For acidic degradation the drug was heated with 5 N HCl at 80°C for 2 h, for alkaline degradation the drug was treated with 5N NaOH at 80°C for 2 h, for degradation under oxidizing conditions the drug was exposed with 10% (v/v) H₂O₂ at room temperature, for thermal degradation the drug solution was exposed at 100°C for 24 h and for heat & moist degradation the drug solution was exposed to 100°C for 48 h. The placebo was also subjected to the same stress conditions to determine whether any peaks arose from the excipients or not. After completion of the treatments the solutions were left to return to room temperature and diluted with water: methanol (40:60, v/v) to furnish 50 µL/mL solutions. The drug was gradually decreased with time on heating at 80°C in 5N HCl, forming major degradation products at RRT 3.94 (Figure 4). Under alkaline condition the drug degrades by the decreased areas in the peaks when compared with peak areas of the same concentration of the non-degraded drug, without giving any additional degradation peaks (Figure 5). Drug degradation was associated with rise in a major degradation product at RRT 3.94 and the drug shows three additional peaks in hydrogen peroxide (30%) degradation at tₘ 5.27, 7.57 and 14.79 min (Figure 6). The drug was stable to thermal condition at 70°C for 72 h and heat and moist condition. The results are shown in.

**Table 4: Assay results for capsules**

<table>
<thead>
<tr>
<th>Capsule formulation</th>
<th>Label claim</th>
<th>Amount of ZHM found</th>
<th>Mean %Recovery</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zipsydron (Sun)</td>
<td>20 mg</td>
<td>19.970</td>
<td>100.314</td>
<td>0.124</td>
</tr>
<tr>
<td>Geodon (Pfizer)</td>
<td>80 mg</td>
<td>80.222</td>
<td>100.278</td>
<td>0.120</td>
</tr>
</tbody>
</table>

**CONCLUSION**
A rapid, specific gradient HPLC method has been developed for the determination of Ezetimibe using UV detector. The method was validated for its performance parameters such as specificity (placebo interference), linearity, system suitability, and accuracy, precision and stability. The method also separates Ziprasidone and also separates Ziprasidone in bulk and capsules from different manufacturers were evaluated for the amount of ZHM in the formulations. Each sample was injected six times after extracting the drug as mentioned above in experimental section. The amount of ZHM was found to be within the range of 99.85%-100.1%. None of the capsule excipients was found to interfere with the analyte peak and the results were shown in Table no 4.

**REFERENCES**


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